SUSCEPTIBLE AND RESISTANT PHASES OF THE DIVIDING SEA-URCHIN EGG WHEN SUBJECTED TO VARIOUS CONCENTRATIONS OF LIPOID-SOLUBLE SUBSTANCES, ESPECIALLY THE HIGHER ALCOHOLS.

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Introduction.

That dividing *Arbacia* eggs show periods of varying susceptibility and resistance when exposed to chemical substances and to various physical conditions has been proved by numerous investigations. When eggs, from a single fertilized lot were placed at regular successive intervals after fertilization in cyanide-containing sea-water (*m*/100 to *m*/200), Lyon[2] found that they were highly resistant to poisoning fifteen or twenty minutes after fertilization, while eggs exposed to the same solution at the time of cytoplasmic division were promptly killed. Later, after the first division had been completed, the resistance to poisoning again returned, followed by a second susceptible period at the second cleavage. Loeb[3] later noted that the unfertilized eggs show greater resistance to cyanide poisoning than the fertilized eggs, and Mathews[4] indicated that in dividing eggs, the period of maximum susceptibility is "immediately before and during segmentation," and that just after segmentation the egg becomes relatively highly resistant. Similar results were obtained by Spaulding[5] in experiments with weak solutions of ether (1/64 per cent. in sea-water). The period of high resistance continued up to the beginning of the first cleavage, and then fell during cleavage to zero, with a sharp rise immediately afterwards. There was a short period of susceptibility immediately following

[1] From the Marine Biological Laboratory, Woods Hole, Mass., and the Department of Zoölogy, Iowa State College, Ames, Iowa.

[2] E. P. Lyon, *Amer. Jour. Physiol.*, 1902, Vol. 7, p. 56.

[3] J. Loeb, *Biochem. Zeitschr.*, 1906, Vol. 1, p. 200.

[4] A. P. Mathews, Biol. Bull., 1906, Vol. 11, p. 137.

[5] E. G. Spaulding, Biol. Bull., 1904, Vol. 6, p. 224.

fertilization. He found also in acid and salt solutions (pure isotonic KCL and NaCL) a similar but less clearly defined rhythm of susceptibility. Eggs subjected to heat, electrical stimulation and hypertonic sea-water behave in a similar manner. Thus, Lyon,[1] observed that the eggs were most resistant to heat at a time previous to the first cleavage, and were most readily injured at the time of division. A. R. Moore[2] finds that the resistance to hypertonic sea-water is least "immediately before and during each cytoplasmic division, and that the maximal resistance is shown 35 to 45 minutes after fertilization and just after each division." More recently Lillie[3] (1916), has made an extensive study of the rhythmical changes in the resistance of the dividing sea-urchin egg to hypotonic sea-water, and has discussed the physiological significance of this rhythm. His experiments show clearly that at or about the time of formation of the cleavage furrow, a marked decline takes place in the resistance of the egg to hypotony, and cytolysis is then rapid and complete. After the cleavage furrow is fully formed the original resistance returns. A similar reversible decline of resistance takes place at the second and third cleavage, and is probably general for mitotic cell-division. The minimum of resistance is found during the formation of the furrow. Both the decline and the return of resistance are rapid, the greater part of each phase occupying four to five minutes. Some increase of susceptibility is apparent ten or twelve minutes before the first appearance of the furrow. Similar observations have been made by Herlant[4] in the egg of *Paracentrotus lividus*.

From such experiments it appears that the resistance of the eggs to a variety of injurious agencies is least at the time when they are undergoing rapid change of form. To account for these rhythmical changes in the physiological state of the egg, Lillie[4] (1909) puts forward the hypothesis that they are essentially the result of variations in the physical condition, especially the permeability, of the surface-film of plasma-membrane, the latter

[1] E. P. Lyon, *Amer. Jour. Physiol.*, 1904, Vol. 11, p. 52.

[2] A. R. Moore, Biol. Bull., 1915, Vol. 28, p. 257.

[3] R. S. Lillie, *Jour. Exper. Zoöl.*, 1916, Vol. 21, No. 3, p. 401.

[4] M. Herlant, *Comptes rendus d. l. Societe d. Biologie*, 1918, Vol. 81, p. 151.

[5] R. S. Lillie, Biol. Bull., 1909, Vol. 17, p. 207.

undergoing a reversible increase in permeability at the time of cleavage. If a rhythm of alternate increase and decrease of permeability accompanies the rhythm of the mitotic process, it seems logical to infer that the entrance of solutes into the cell would occur most readily when there is a loss of semi-permeability. Accompanying this change would be a decrease of the electrical surface-polarization, and this in turn probably would alter the metabolic processes, especially oxidations within the cell. Cell metabolism then is inseparably bound up with cell-permeability; and the plasma-membrane, or semi-permeable surface-layer is something more than a haptogen membrane (to which it has frequently been compared). In discussing this subject in a later paper, Lillie[1] makes it especially clear that this "general characteristic of semi-permeability (the all-essential insulating and diffusing-preventing property) is not merely the result of a special chemical composition and structural density, such as determine the semi-permeability of a precipitation-membrane, but is inseparable from the living condition, *i.e.*, is actively maintained by a continual process of metabolism. The proof of this is that death—the cessation of metabolism—however caused, is invariably followed by a loss of semi-permeability, *i.e.*, the normal state of the membrane then ceases to be maintained and the unhindered processes of diffusion lead to the disintegration of the cell. Hence destruction of the surface-layer by artificial means—cytolytic substances, heat, extensive mechanical injury—is quickly fatal to all cells."

In the experiments about to be described, I have studied the behavior of fertilized Arbacia eggs when subjected for definite brief lengths of time to various concentrations of some of the higher alcohols—anyl, hexyl, heptyl, octyl and capryl—at different periods of the cell-division cycle. This work was undertaken at the Marine Biological Laboratory, at Woods Hole, Mass., during the past summer at the suggestion of Professor Ralph Lillie, to whom the writer expresses his hearty thanks for many kind suggestions and directions during its prosecution.

[1] R. S. Lillie, *Amer. Journ. Physiol.*, 1918, Vol. 45, No. 4, p. 406.

Experimentation.

In order to procure a sufficient number of eggs for each series of experiments, between one and two dozen large females were opened, and their eggs collected into finger bowls. By successive washing and settling, a uniform mass of mature eggs was obtained, which could be inseminated and divided into two parts; one to be used for the control, and the other for the experiments. It was found early in the work that the success of the experiments depended upon having batches of eggs which were sufficiently mature and uniform, so that all eggs reached successive stages in their development at practically the same time. It was also found that great exactness in the time-relations of the operations was absolutely essential, and that any variation once entered upon was sufficient to make the results worthless from a comparative standpoint. Usually two series of experiments were started in a day; one in the morning to be carried over to the gastrula stage by the following morning, and one in the afternoon, to be examined the following afternoon. After extended preliminary experimentation, it was found convenient, in any one series, to keep the time of exposure constant and to vary the concentration of substance used, although in a considerable number of experiments the opposite procedure was adopted, *i.e.*, the time was varied and the concentration kept constant.

Practically the same procedure was observed throughout the entire experimentation. At each of the successive intervals after fertilization, usually ten minute intervals, about one half of medicine pipette containing a suspension of the inseminated eggs was placed in a small corked Erlenmeyer flask, containing 50 c.c. of the solution of the alcohol in sea-water, and allowed to remain for the time of exposure chosen (usually five minutes). After the given time had nearly elapsed, the excess liquid was poured off, and the eggs with a little of the liquid were placed in a watch glass and the immediate results of the treatment were observed under the low power of the microscope. At the termination of the time of exposure, the watch glass containing the eggs was carefully immersed in a large volume of sea-water in a finger bowl and the water was changed several times to rid it of the excess substance. Finally the eggs were very carefully

washed with a stream of water from the medicine-dropper, and set aside to undergo development. The proportion proceeding with development to the free-swimming larval stage was subsequently determined. It was found that the estimate of the proportion surviving to the blastula stage was more readily and exactly made, if the watch glass containing the eggs was removed from the bowl of sea-water just before the free-swimming larval stage was reached. Thus all survivors could be confined within a small volume, and the count or estimate easily made. As a rule, the experiments were carried only up to about the time of second cleavage; since the evidence indicates that the same variation of susceptibility occurs in each cell division cycle; moreover divergencies between the different eggs in any lot become more pronounced as time elapses, and it is important that all eggs of a lot should be in the same physiological state at the time of treatment.

At first several preliminary experiments were necessary in order to determine the most suitable range of concentrations to be used, since the time of exposures determined upon were brief, the longest being ten minutes; in some cases of exposures only three minutes were used. In this connection, the tables given by Lillie[1] in his paper on the action of various anæsthetics in suppressing cell-division in sea-urchin eggs, were exceedingly helpful. For i-Amyl[2] alcohol, he finds 0.45 to 0.4 vol. per cent. a favorable anæsthetic concentration for eggs subjected for two and one half hours, while 0.5 vol. per cent. and above are somewhat rapidly toxic. For Capryl[3] alcohol he finds the anæsthetizing concentrations to range between 0.012 and 0.02, and notes that even in sub-anæsthetic concentrations this alcohol exhibits a relatively high specific toxicity. With the help of these data, and also Fühner's[4] observations showing that in a series of monohydric aliphatic alcohols each member of the group is from three to four times as effective (for equimolecular concentrations)[5] as its immediate predecessor, it became a compara-

[1] R. S. Lillie, *Journ. Biolog. Chem.*, 1914, Vol. 17, No. 2, pp. 129–139.

[2] Cf. reference just cited; Table VIII., p. 135.

[3] Cf. reference just cited; Table IX., p. 137.

[4] H. Fühner, *Arch. f. exp. Path. u. Pharm.*, 1904, LII., p. 69.

[5] Capryl alcohol used in exposures of five minutes seemed not to obey this general rule, since in practically all experiments it was used in concentrations nearly three times its computed strength. (See p. 137.)

tively easy matter to approximate the most suitable concentration of each alcohol after the first had been determined.

Amyl Alcohol.

Summarizing briefly the results of preliminary observations it was found that the most satisfactory concentration of i-Amyl alcohol when used with exposures of three to eight minutes, was between 0.7 and 0.9 vol. per cent. Solutions of this strength are sufficiently toxic to prevent many but not all of the eggs thus treated from developing to a larval stage. Solutions weaker than 0.7 vol. per cent. permit practically all eggs to proceed to

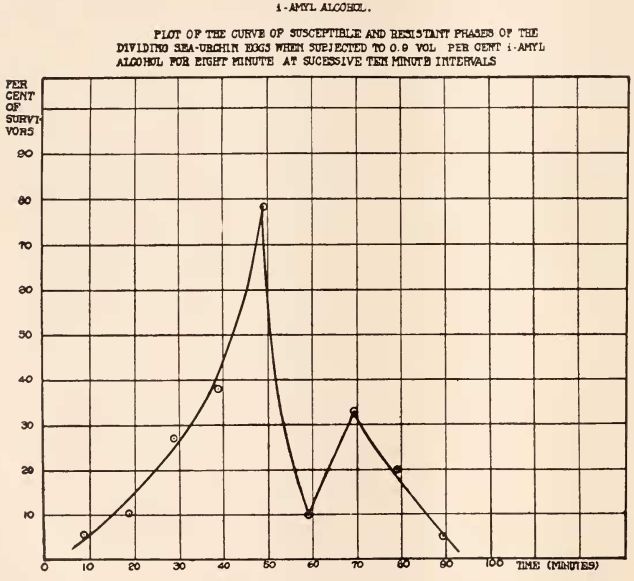

Fig. 1.

the blastula stage with these exposures, with little appreciable difference. At 1.0 vol. per cent. not more than 20 per cent. of eggs form free-swimming blastulæ even when exposed at the period of highest resistance; (*e.g.*, 30 to 40 minutes after fertili-

zation) and above this concentration the toxicity is such that the decline in survivals is very rapid. In 1.25 vol. per cent. solutions, all eggs are killed in all stages with exposures of nine minutes.

Table I. summarizes the results of a typical series of experiments with i-amyl alcohol. This particular series of experiments was started in the afternoon of July 16, and the observations noted in the third column were carried over into the morning of

Table I.

I-Amyl Alcohol.

July 16, 1:45 P.M. The fertilized eggs were placed at the intervals after fertilization noted in column 1 in 50 cc. of 0.9 vol. per cent i-amyl alcohol. Al exposures except the first (1) (6 minutes) were of eight minutes duration.

Intervals After Fertilization.	Observed Condition of the Eggs at the Time of Removal from Sol.	Proportion Forming Blastulæ and Condition of Remaining Eggs Next Day.
(1) 3– 9 m.	Fertilization-membranes well formed. No marked cytolytic change noted.	About 5 per cent. form free swimming blastulæ. Considerable numbers cytolyzed.
(2) 10–18 m.	No marked change in appearance. Uniform.	About 10 per cent. form blastulæ. Not so badly cytolyzed; most cells intact.
(3) 20–28 m.	Membranes markedly swollen in some cases. Slight fading of pigment.	Nearly 30 per cent. free-swimming blastulæ. Most eggs intact.
(4) 30–38 m.	A few cells plasmolyzed, other membranes markedly swollen.	Between 30 and 40 per cent. free-swimming blastulæ. Most others intact but swollen.
(5) 40–48 m.	No marked change. Faint indication of cleavage furrow in a few scattered cells.	Large majority (75–80 per cent.) form swimming blastulæ.
(6) 50–58 m.	About 65 per cent. have entered the two-celled stage. Some show shrinkage.	Relatively few (less than 10 per cent.) form surviving blastulæ.
(7) 60–68 m.	About 90 per cent. in two-celled stage. Others intact.	Between 30–35 per cent. form blastulæ.
(8) 70–78 m.	Practically all in two-celled stage.	Few (15–20 per cent.) form blastulæ. Others intact.
(9) 80–88 m.	A few are starting second cleavage furrow.	3–5 per cent. form blastulæ. Others intact.

the following day. A similar series of experiments performed at about the same time with the same alcohol in somewhat lower concentration (0.8 vol. per cent.), but with slightly longer (10-minute) exposures yielded substantially the same results. On the following day experiments were carried out on eggs subjected to 1.1 vol. per cent. solutions with only brief (3-, 4- and 5-minute) exposures with the results noted above.

In the controls about one half of the eggs were in the two-celled stage at fifty-three minutes after fertilization, and at sixty-five minutes between 85 and 90 per cent. were divided. There is a definite period of well-marked susceptibility immediately following fertilization; the susceptibility then gradually and progressively declines up to the end of forty-eight minutes (just before the first cleavage). There then follows a very susceptible period just at the time of cleavage. Later the resistant phase reappears until about the time of second cleavage. If the time intervals are plotted as abscissæ, and the percentage of surviving blastulæ as ordinates, the relationships may be represented in the curve shown in Fig. 1.

Hexyl Alcohol.

In exploring the range of suitable concentrations for hexyl alcohol, the next higher member of the series, assuming that it should be approximately three times as effective as i-amyl alcohol, three preliminary experiments were performed. For these, solutions of 0.1, 0.25 and 0.30 vol. per cent. were used respectively. The time of exposure was shortened to five minutes, for the reason that it was thought the concentrations were, if anything, a little above the optimum. The results clearly showed that the solutions of 0.1 vol. per cent. was not sufficiently toxic to demonstrate any variation of susceptibility in the eggs, since at whatever period they were exposed practically all eggs survived to the free-swimming blastula stage. On the other hand, the two higher concentrations proved too toxic, so that practically none of the eggs continued their development after subjection to these solutions at any period. The 0.25 vol. per cent. solution, although it suppressed further development, was not quite intense enough in its action to cause cytolysis in the eggs, with few exceptions. The 0.30 vol. per cent. concentration caused very evident cytolysis, and rupture was almost universal. Accordingly, series of experiments were carried out to test the various concentrations between 0.1 vol. per cent. and 0.25 vol. per cent. Two of these experiments are summarized in Table II., and may be regarded as typical.

These results show a much less definite evidence of a rhythm of

TABLE II.

HEXYL ALCOHOL.

August 11, 10:15 A.M. Fertilized Arbacia eggs were placed at intervals noted in 50 cc. of 0.13 and 0.17 vol. per cent. of Hexyl alcohol respectively, and allowed to remain in them for five minutes. They were then placed in watch glasses, quickly observed, and treated as described in the previous experiment.

Intervals After Fertilization.	(A) 0.13 Vol. Per Cent.		(B) 0.17 Vol. Per Cent.	
	Observed Condition.	Proportion Forming Blastulæ.	Observed Condition.	Proportion Forming Blastulæ.
(1) 15–20m.	Fert. membrane well formed. No marked cytolysis noted.	Majority (90 per cent.) form blastulæ.	No marked cytolysis. Uniform batch of fertile eggs.	Between 70 and 80 per cent. form blastulæ. Other cytolyzed but intact.
(2) 25–30m.	No marked change.	Between 85–90 per cent. form swimming blastulæ	Fert. membrane in most eggs swollen; no great change otherwise.	70–75 per cent. blastulæ. Few ruptured.
(3) 35–40m.	Membrane swollen, few show slight plasmolysis.	Practically all (90 per cent. or over) form blastulæ. Others intact.	No marked change noted.	90 per cent. form blastulæ.
(4) 45–50m.	Slight loss in pigmentation. No marked cytolysis however.	90 per cent. form blastulæ. Few ruptured.	Slight fading of pigment. No other marked change.	Between 65–70 per cent. form blastulæ. Few scattered cells ruptured.
(5) 55–60m.	About half in two-celled stage. No marked change.	80–85 per cent. form blastulæ. Others mostly intact.	All intact, 50 per cent. or over in two-celled stage.	Nearly 60 per cent. form blastulæ.
(6) 65–70m.	Fully 85 per cent. in two-celled stage; no marked change from preceding.	About 76 per cent. form swimming blastulæ.	No change.	Not more than 50 per cent. form blastulæ. Others badly cytolyzed but mostly intact.
(7) 75–80m.	No marked change.	Between 65–70 per cent. swimming blastulæ; others badly cytolyzed.	Slight loss of pigment. No marked change.	About 60 per cent. blastulæ. Two-celled eggs conspicuous. Most others intact.
(8) 85–90m.	Few cells (ca. 5–7 per cent.) show second cleavage. No marked change.	About 60 per cent. form swimming blastulæ. Most others intact.	Few cells in second cleavage. No marked cytolysis.	Nearly 85 per cent. swimming blastulæ.

susceptibility than those just described for i-amyl alcohol. The eggs apparently maintain throughout the cycle a relatively high resistance to the concentrations of hexyl alcohol here used, with only a slight increase of susceptibility at the time of

first cleavage; in the one case (0.17 vol. per cent.) there is evidence of a slight return of resistance just afterwards, and in the other (0.13 vol. per cent.) there is not. Why there should be this difference in the behavior of the two alcohols is difficult to explain. The exposures to hexyl alcohol were perhaps insufficiently

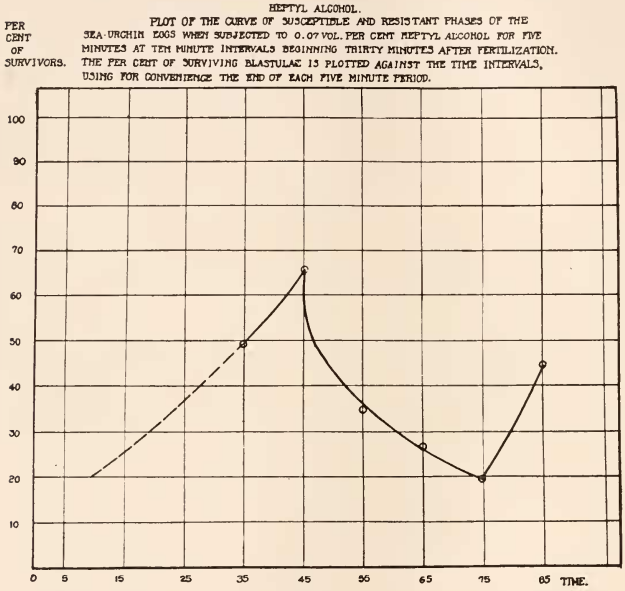

FIG. 2.

prolonged to bring out a well marked differential effect at the different stages of the cycle. Again, it is well known that certain anæsthetics are less effective than others in suppressing the cell-division process; also many neuromuscular responses react differently to a given anæsthetic in different animals, and in the same animal at different ages. Thus Lillie[1] found chloretone much less effective than chloral hydrate in suppressing cleavage in Arbacia eggs. As regards the alcohols that he tried, he lists propyl, butyl, amyl in the order of increasing favorability, while

[1] R. S. Lillie, *Jour. Biol. Chem.*, 1914, Vol. 17, No. 2, p. 130.

ethyl and capryl show a higher toxicity than the others.[1] It may be that differences,—both qualitative and quantitative—in the lipoid elements of the tissues, and hence in the plasmamembrane, form the basis of the observed physiological difference.

In the controls which were running parallel to the two experiments just described, a majority of the eggs entered the two-celled stage at about fifty-eight minutes after fertilization. At sixty-five minutes after fertilization, between 85 and 90 per cent. had cleaved. Practically all eggs in the controls had reached the blastula stage the following day.

Heptyl Alcohol.

A series of six experiments were performed with this alcohol to determine the limits of suitable concentration. Reasoning from the data in the proceeding, it was thought that the optimum concentration would be in the proximity of between 0.04 and 0.07 vol. per cent., but to make sure, concentrations as low as 0.02 and as high as 0.08 vol. per cent. were used. As a matter of fact, solutions of 0.06 and 0.07 vol. per cent. were found to be the best suited to the experiments, although it is interesting to note that even weaker solutions showed marked toxic action on eggs at the time of formation of the first cleavage furrow, while during the stages preceding and succeeding division, they had relatively little influence. Table III. summarizes three experiments with this alcohol, and is fairly typical of results obtained in other experiments. By some unavoidable oversight in technical procedure, records for the first two periods (*i.e.*, respectively 10 and 20 minutes after fertilization) were not obtained, but from the results of other series it is evident that the eggs maintain a rather high resistance at these times. The results given in Table III. show that 0.07 vol. per cent. approximates the favorable concentration for this alcohol, while the solutions on either side are slightly hypo- and hyper-toxic respectively; *i.e.*, in the one case practically all eggs survive to the blastula stage, and in the other nearly all die. Recovery of resistance after the first division appears to be relatively slow. When the percentage of surviving blastulæ is plotted against the time intervals, regarding

[2] See *ibid.*, p. 133.

TABLE III.

HEPTYL ALCOHOL.

Fertilized *Arbacia* eggs were placed in 50 cc.. of the following solutions of heptyl alcohol at ten minute intervals, and allowed to remain in each solution (0.06, 0.07 and 0.08 vol. per cent.) for five minutes. They were then treated as previously described.

Intervals.	(A) 0.06.		(B) 0.07.		(C) 0.08.	
	Observed Condition.	No. Blast.	Observed Condition.	No. Blast.	Observed Condition.	No. Blast.
(1) 30–35 m.	No marked cytolysis. Membranes slightly swollen.	70–75 per cent. free-swimming blastulæ. Few ruptured.	Membranes slightly swollen. No appreciable loss of pigment.	About 48–50 per cent. swimming blastulæ. Other cells ruptured in many cases.	Noticeable loss of pigment. Membranes markedly swollen, cytoplasm shows slight cretination.	Not more than 1 per cent. swimming blastulæ Majority intact.
(2) 40–45 m.	No marked change.	About 82 per cent. swimming blastulæ. Others intact.	Slight loss pigment. No other marked change.	Nearly 65 per cent. swimming blastulæ. Few ruptured.	About as noted above.	Practically no swimming blastulæ. Most badly cytolyzed.
(3) 50–55 m.	Between 25–30 per cent. show furrow. Some show loss of pigment.	88–90 per cent. active. Some ruptured but still active.	No marked change.	Relatively few (33–35) per cent. active. Whole masses of cells ruptured. None of two-celled stage stage seen in field. seen in field.	Marked loss of pigment. All intact however.	Less than 1 per cent. active. Others badly cytolyzed but mostly intact.
(4) 60–65 m.	About 80 per cent. in or entering two-celled stage. No great changes noted.	About 75 per cent. active. Considerable number remain in two-celled stage but are intact.	Nearly 80 per cent. in cleavage. Rather noticeable loss of pigment. All intact.	Between 24–28 per cent. active blastulæ. Marked cytolysis, with all stages of rupturing. Rather striking condition.	Marked loss of pigment. Majority cleaving.	Only a few survivors. Others ruptured, and none of the two-celled eggs intact.

TABLE III.—*Continued.*

Intervals.	(A) 0.06.		(B) 0.07.		(C) 0.08.	
	Observed Condition.	No. Blast.	Observed Condition.	No. Blast.	Observed Condition.	No. Blast.
(5) 70–75 m.	Majority have cleaved, no marked cytolysis noted.	Between 85–90 per cent. active. A few cells are ruptured. Others rather badly cytolyzed.	90 per cent. two-celled. Slight loss of pigment noted.	Not over 20 per cent. active. Most others badly cytolyzed, a few ruptured.	Majority as before in two-celled stage. Pigment faded.	All badly cytolyzed, over half ruptured.
(6) 80–85 m.	Considerable number show second furrow.	Few active blastulæ. Large number ruptured but most two-celled eggs intact.	No marked change.	Between 40–50 per cent. active glastulæ. Others intact although badly cytolyzed.	Pigment loss characteristic. No other marked cytolytic change.	About 5 per cent. active blastulæ. Others ruptured.

as typical the data of the 0.07 vol. per cent. solution, an interesting curve is obtained (Fig. 2) which is fairly comparable with the one shown for i-amyl alcohol. There is a gradual rise in resistance up to the period of first cleavage, with a sharp drop during cell-division followed by a slow recoverry.

Octyl Alcohol.

Normal octyl alcohol is apparently considerably more toxic than its isomere capryl alcohol. In a series of five experiments with octyl alcohol in concentrations ranging from 0.010 to 0.030 vol. per cent., the best concentration for five minute times of exposure was found to be in the neighborhood of 0.015 vol. per cent. On the other hand the outcome of fourteen experiments with capryl alcohol showed the optimum concentration for the same time of exposure to be between 0.035 and 0.045 vol. per cent., which is between two and three times the favorable concentration of normal octyl alcohol. Table IV. summaries a

Table IV.

Normal Octyl Alcohol.

Fertilized eggs were subjected for five minutes to 0.013 vol. per cent. of normal octyl alcohol at intervals of ten minutes.

Intervals After Fertilization.	Observed Condition on Removal from Fluid.	Observed Condition the Following Day.
(1) 15–20 m.	Fertilization membrane well formed. Slight loss of pigment.	Nearly 50 per cent. active blastulæ. Others badly cytolyzed, few ruptured.
(2) 25–30 m.	No noticeable cytolysis although very marked loss of pigment.	About 65 per cent. active blastulæ. Others cytolyzed.
(3) 35–40 m.	Decided loss of pigment. No marked change in membrane or cytoplasm.	Nearly 80 per cent. active. Others intact.
(4) 45–50 m.	About 2 per cent. show first furrow. Slight loss pigment. No cytolysis.	Practically all active blastulæ.
(5) 55–60 m.	Over half in first cleavage.	About 85 per cent. active.
(6) 65–70 m.	About 90 per cent. in two-celled stage.	Almost 60 per cent. active blastulæ, numbers of two-celled egg present and mostly intact. Some badly cytolyzed and ruptured.
(7) 75–80 m.	Aside from loss of pigment no noticeable change.	Between 65 and 70 per cent. active blastulæ. Most others cytolyzed but intact.
(8) 85–90 m.	A few (1 per cent.) just begin to show second cleavage furrow. No marked change in appearance.	Between 85 and 90 per cent. active blastulæ. Others cytolyzed but intact.

typical experiment using normal octyl alcohol of 0.013 vol. per cent. concentration. For exposures of five minutes duration, this concentration gave the best results, and showed very clearly the resistant and susceptible phases.

Capryl Alcohol.

As mentioned before, experiments with various concentrations of capryl alcohol showed that for brief exposures, the most favorable concentration was nearly three times that of normal octyl alcohol. This may perhaps be accounted for in some measure by the fact that not all samples of capryl alcohol are

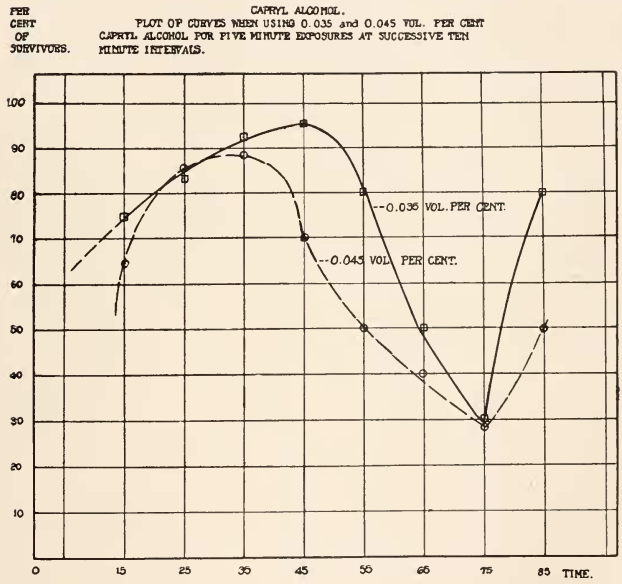

Fig. 3.

uniform in chemical composition and purity; a slight difference in this respect is known to make a decided difference in its chemical and physiological activity. In suitable concentrations, this alcohol is without doubt one of the most satisfactory for

showing susceptible and resistant phases in dividing eggs. Several experiments were tried with this alcohol in which the concentration was kept constant and the time of exposure was varied; and from the data thus gathered, it seems probable that of the two factors, concentration is the more important. In other words, if the concentration is such that it gives the best

Table V.

Fertilized eggs were subjected for five minutes at ten minute intervals to 0.035 and 0.045 vol. per cent. capryl alcohol.

Intervals.	(A) 0.035 Vol. Per Cent.		(B) 0.045 Vol. Per Cent.	
	Observed Condition.	Condition Following Day.	Observed Condition.	Condition Following Day.
(1) 10–15m.	Membranes well formed. No great difference from normal eggs.	75–80 per cent. active. Others cytolyzed but mostly intact.	No marked difference from normal eggs.	About 65 per cent active. Others badly cytolyzed.
(2) 20–25m.	Membrane slightly swollen. No marked cytolysis.	About 83 per cent. active. Others badly cytolyzed.	No marked change.	Nearly 85 per cent. active. Others intact.
(3) 30–35m.	Few show loss of pigment, cytoplasm shrunken in some cases.	About 92 per cent. active. Others intact.	Marked fading of pigment. No marked cytolysis.	Between 85–90 per cent. active. Some ruptured eggs motile.
(4) 40–45m.	No marked change. None show cleavage furrow.	About 95 per cent. active blastulæ. Others intact.	No marked change, slight loss of pigment.	About 70 per cent active. Others mostly intact.
(5) 50–55m.	Nearly half show first cleavage furrow No marked change.	80 per cent. active blastulæ. Few ruptured.	No marked change.	About 50 per cent. active blastulæ. Most others badly cytolyzed, many ruptured.
(6) 60–65m.	Nearly all show first cleavage furrow. No marked cytolysis.	About 50 per cent. active blastulæ. Most others ruptured.	Practically all in two cell stage. Some loss pigment.	About 40 per cent. active. All others badly ruptured. Some still in two cells.
(7) 70–75m.	No marked change. Few scattered cells show second furrow.	About 30 per cent. active blastulæ. Others badly cytolyzed.	No marked change, except loss of pigment.	Nearly 30 per cent. active. Others badly cytolyzed. Few persist in two cells.
(8) 80–85m.	No marked cytolysis.	Nearly 80 per cent. active blastulæ. Others mostly intact.	No marked change.	Nearly 50 per cent. active blastulæ. Others cytolyzed but mostly intact.

When plotted the data gives interesting curves as shown in Fig. 3.

results with a five-minute exposure, when the exposure is prolonged to eight minutes, very little or no difference is detected. This generalization, however, could probably be applied only within narrow limits.

The data from two experiments using capryl alcohol in 0.035 and 0.045 vol. per cent. concentrations respectively are given in Table V. These records are fairly typical of results of other experiments.

Summary.

1. The developing sea-urchin egg when subjected to suitable concentrations of various lipoid-soluble substances—i-amyl, hexyl, heptyl, octyl and capryl alcohols—shows unmistakable rhythms of susceptible and resistant phases, which when taken in connection with the earlier observations of Lyon, Herlant, Mathews, Spaulding, Lillie and others, constitute additional evidence that a very intimate relation exists between the general physiological condition of the egg, and the physical state of its plasma-membrane.

2. During the first ten or fifteen minutes after fertilization the eggs are more susceptible than at any other time until the period just preceding division. A comparatively resistant phase gradually becomes more and more marked up to just before the first cell-division (about 45 or 48 minutes after fertilization). This is followed by a period of decidedly increased susceptibility which lasts for about 15 or 20 minutes, during which time marked cytological effects are noted. Subsequently the resistant phase is largely recovered, and maintained up to the time of the second cleavage.

3. The most favorable concentrations of the various alcohols for demonstrating the rhythm of susceptibility range as follows: i-amyl, between 0.7 and 0.9 vol. per cent.; hexyl, between 0.13 and 0.17 vol. per cent.; heptyl, between 0.06 and 0.07 vol. per cent.; normal octyl, about 0.015; while capryl was considerably above its isomere (normal octyl) between 0.035 and 0.045 vol. per cent. The best records were obtained in experiments using i-amyl and capryl alcohols, possibly indicating a higher specific toxicity of these when compared to the others.

4. When suitable concentrations were used, no marked

differences could be detected by varying slightly the durations of exposure. Eggs exposed for five, eight or even ten minutes to the same concentration gave similar results. This, however, would probably apply only within narrow limits.